Interplay between Velocity and Travel Distance of Kinesin-based Transport in the Presence of Tau

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ABSTRACT Although the disease-relevant microtubule-associated protein tau is known to severely inhibit kinesin-based transport in vitro, the potential mechanisms for reversing this detrimental effect to maintain healthy transport in cells remain unknown. Here we report the unambiguous upregulation of multiple-kinesin travel distance despite the presence of tau, via decreased single-kinesin velocity. Interestingly, the presence of tau also modestly reduced cargo velocity in multiple-kinesin transport, and our stochastic simulations indicate that the tau-mediated reduction in single-kinesin travel underlies this observation. Taken together, our observations highlight a nontrivial interplay between velocity and travel distance for kinesin transport, and suggest that single-kinesin velocity is a promising experimental handle for tuning the effect of tau on multiple-kinesin travel distance.

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Conventional kinesin is a major microtubule-based molecular motor that enables long-range transport in living cells. Although traditionally investigated in the context of single-motor experiments, two or more kinesin motors are often linked together to transport the same cargo in vivo (1–4). Understanding the control and regulation of the group function of multiple kinesins has important implications for reversing failure modes of transport in a variety of human diseases, particularly neurodegenerative diseases. Tau is a disease-relevant protein enriched in neurons (5,6). The decoration of microtubules with tau is known to strongly inhibit kinesin transport in vitro (7–9), but how kinesin-based transport is maintained in the presence of high levels of tau, particularly in healthy neurons, remains an important open question. To date, no mechanism has been directly demonstrated to reverse the inhibitory effect of tau on kinesin-based transport. Here we present a simple in vitro study that demonstrates the significant upregulation of multiple-kinesin travel distance with decreasing ATP concentration, despite the presence of tau.

This investigation was motivated by our recent finding that single-kinesin velocity is a key controller for multiple-kinesin travel distance along bare microtubules (10). The active stepping of each kinesin motor is stimulated by ATP (11), and each kinesin motor remains strongly bound to the microtubule between successive steps (10,11). As demonstrated for bare microtubules (10), with decreasing ATP concentrations, each microtubule-bound kinesin experiences a decreased stepping rate per unit time and spends an increased fraction of time in the strongly bound state; additional unbound kinesins on the same cargo have more time to bind to the microtubule before cargo travel terminates. Thus, reductions in single-kinesin velocity increase the probability that at least one kinesin motor will remain bound to the microtubule per unit time, thereby increasing the travel distance of each cargo (10). Because this effect only pertains to the stepping rate of each individual kinesin and does not address the potential presence of roadblocks such as tau on the microtubules, we hypothesized in this study that single-kinesin velocity may be exploited to relieve the impact of tau on multiple-kinesin travel distance.

We focused our in vitro investigation on human tau 23 (htau23, or 3RS tau), an isoform of tau that exhibits the strongest inhibitory effect on kinesin-based transport (7–9). Importantly, htau23 does not alter the stepping rate of individual kinesins (7,9), supporting our hypothesis and enabling us to decouple single-kinesin velocity from the potential effects of tau. We carried out multiple-kinesin motility experiments using polystyrene beads as in vitro cargos (8,10), ATP concentration as an in vitro handle to controllably tune single-kinesin velocity (10,11), and three input kinesin concentrations to test the generality of potential findings for multiple-kinesin transport. Combined with previous two-kinesin studies (10,12), our measurements of travel distance (Fig. 1 *A*) indicate that the lowest kinesin concentration employed (0.8 nM) corresponds to an average of ~2–3 kinesins per cargo. Note that in the absence of tau, the observed decrease in bead velocity at the higher kinesin concentrations (Fig. 1 *A*) is consistent with a recent in vitro finding (13). At 1 mM ATP, htau23 reduced kinesin-based travel distance by a factor of two or more (Fig. 1, *A*

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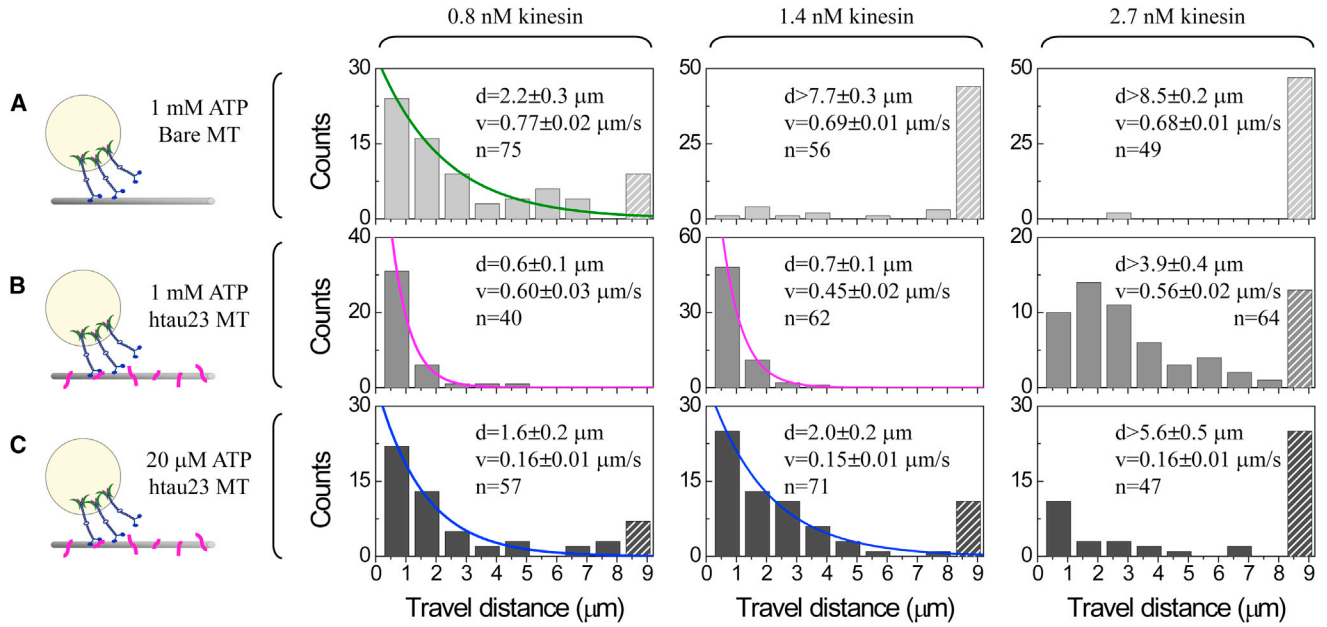

FIGURE 1 Distributions of multiple-kinesin travel distances measured at three experimental conditions, to verify the effect of tau (*A* and *B*) and to investigate the impact of single-kinesin velocity on the tau effect (*B* and *C*). Shaded bars at 8.7 μm indicate counts of travel exceeding the field of view. The mean travel distance (*d*; ± standard error of mean, SEM), sample size (*n*), and corresponding mean velocity (*v*; ± SEM) are indicated. MT denotes microtubule. Mean travel distance increased substantially at 20 μM ATP (*C*), despite the presence of htau23. This effect persisted across all three kinesin concentrations tested (*left* to *right*).

and *B*). This observation is in good agreement with previous reports (7,8).

Consistent with our hypothesis, reducing the available ATP concentration to 20 μM increased the multiple-kinesin travel distance by >1.4-fold for all three input kinesin concentrations (Fig. 1, *B* and *C*), despite the presence of htau23. The corresponding reduction in single-kinesin velocity with decreasing ATP concentration (10,11) is reflected in the ~3.4-fold reduction in the measured bead velocities (Fig. 1, *B* and *C*). Therefore, the strong negative relationship between single-kinesin velocity and multiple-kinesin travel distance occurs not only for bare microtubules (10), but also for tau-decorated microtubules.

What causes the observed increase in travel distance at the lower ATP concentration (Fig. 1, *B* and *C*)? In addition to the mechanism discussed above for the case of bare microtubules (10), an intriguing mechanism was suggested by recent studies of tau-microtubule interactions in which htau23 was observed to dynamically diffuse along microtubule lattices (14,15): reducing the stepping rate of a microtubule-bound kinesin may effectively increase the probability that a tau roadblock can diffuse away before the kinesin takes its next step.

Perhaps surprisingly, although htau23 does not impact single-kinesin velocity (7,9), we observed a modest reduction in the average velocity of multiple-kinesin transport in experiments using tau-decorated microtubules (Fig. 1, *A* and *B*). This decreased velocity reflects a substantially larger variance in the instantaneous velocity for bead trajectories in the presence of htau23 (see Fig. S1 in the Supporting Material), as quantified by parsing each bead trajectory into a series of constant-velocity segments using a previously developed automatic software incorporating Bayesian statistics (16).

To test the possibility that single-kinesin travel distance impacts multiple-kinesin velocity, we performed stochastic simulations (see the Supporting Material) that assumed *N* identical kinesin motors available for transport and included kinesin's detachment kinetics (17). Previously, this model successfully captured multiple-dynein travel distances in vivo using single-dynein characteristics measured in vitro (18). In this study, we introduced one (and only one) free parameter to reflect the probability of each bound kinesin encountering tau at each step. When encountering tau, each kinesin has a 54% probability of detaching from the microtubule (interpolated from Fig. 2A of Dixit et al. (7)); the undetached kinesin is assumed to remain engaged in transport and completes its step along the microtubule despite the presence of tau.

Remarkably, our simple simulation suggested that the tau-mediated reduction in single-kinesin travel is sufficient to reduce multiple-kinesin velocity (Fig. 2 *A*). The majority of the velocity decrease is predicted to occur at the transition from single-kinesin to two-kinesin transport (Fig. 2). Further decreases in cargo velocity with increasing motor number are predicted to be modest and largely independent of tau (Fig. 2 *B*). The results of our simulation remain qualitatively the same when evaluated at two bounds (40 and

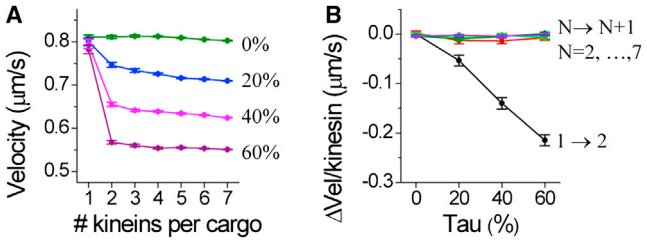

FIGURE 2 Stochastic simulations predict a tau-dependent reduction in multiple-kinesin velocity, assuming that the only effect of tau protein is to prematurely detach kinesin from the microtubule (or, to reduce single-kinesin travel distance). (*A*) Average velocity of cargos carried by the indicated number of kinesins was evaluated at 1 mM ATP, and for four probabilities that a kinesin may encounter tau at each step. Mean velocity was evaluated using 600 simulated trajectories for all simulation conditions. Error bars indicate SEM. (*B*) Change in cargo velocity with each additional kinesin (ΔVel/kinesin) as a function of tau-encounter probability. These values were calculated from cargo velocities shown in panel *A*. Error bars indicate SEM.

65%) encompassing the interpolated 54% probability of kinesin detaching at tau (see Fig. S2).

We note that our simple simulations do not consider the possibility that kinesin may pause in front of a tau roadblock, as previously reported in Dixit et al. (7). We omitted this consideration because the interaction strength between kinesin and the microtubule in such a paused state is unknown. In a multiple-motor geometry, could a paused kinesin be dragged along by the other motors bound to the same cargo? Could a tau roadblock be forcefully swept off the microtubule surface by the collective motion of the cargo-motor complex? Significant experimental innovations are necessary to specifically address these questions in future multiple-motor assays and to guide modeling efforts. Nonetheless, our simple simulation demonstrates that reducing single-kinesin travel distance is sufficient to decrease multiple-kinesin travel distance.

Taken together, our observations highlight a nontrivial interplay between velocity and travel distance for kinesin-based transport in the presence of tau. We uncover a previously unexplored dual inhibition of tau on kinesin-transport: in addition to limiting cargo travel distance, the tau-mediated reduction in single-kinesin travel distance also leads to a modest reduction in multiple-kinesin velocity. We provide what we believe to be the first demonstration of the unambiguous upregulation of multiple-kinesin travel distance despite the presence of tau, via reducing single-kinesin velocity, suggesting a mechanism that could be harnessed for future therapeutic interventions in diseases that result from aberrant kinesin-based transport.

SUPPORTING MATERIAL

Two figures and Materials and Methods information are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(13)01134-X.

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